Different frequencies of repetitive transcranial magnetic stimulation combined with local injection of botulinum toxin type A for post-stroke lower limb spasticity: study protocol for a prospective, single-center, non-randomized, controlled clinical trial

https://doi.org/10.4103/1673-5374.339011

Date of submission: November 10, 2021

Date of decision: November 26, 2021

Date of acceptance: December 20, 2021

Date of web publication: April 1, 2022

Yang Shao, Yang Yang, Yong-Xin Sun, Ai-Hua Xu*

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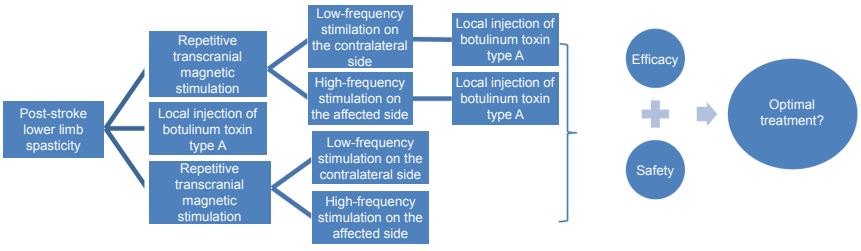
Graphical Abstract *Repetitive transcranial magnetic stimulation for post-stroke lower limb spasticity*



Abstract

No definite consensus has currently been reached regarding the safety and efficacy of low- or high-frequency repetitive transcranial magnetic stimulation in the treatment of post-stroke muscle spasticity. The latest research indicates that when combined with local injections of botulinum toxin type A, it is more effective on post-stroke muscle spasticity than local injections of botulinum toxin type A alone. We designed a prospective, single-center, non-randomized, controlled clinical trial to investigate the safety and efficacy of different frequencies of repetitive transcranial magnetic stimulation combined with local injections of botulinum toxin type A in treating post-stroke lower limb muscle spasticity to determine an optimal therapeutic regimen. This trial will enroll 150 patients with post-stroke muscle spasticity admitted to the Department of Rehabilitation Medicine at the First Affiliated Hospital of China Medical University. All enrolled patients will undergo routine rehabilitation training and will be divided into five groups (n = 30 per group) according to the particular area of cerebral infarction and treatment methods. Group A: Patients with massive cerebral infarction will be given local injections of botulinum toxin type A and low-frequency (1 Hz) repetitive transcranial magnetic stimulation on the contralateral side; Group B: Patients with non-massive cerebral infarction will be given local injections of botulinum toxin type A and high-frequency (10–20 Hz) repetitive transcranial magnetic stimulation on the affected side; Group C: Patients with massive/non-massive cerebral infarction will be given local injections of botulinum toxin type A; Group D: Patients with massive cerebral infarction will be given low-frequency (1 Hz) repetitive transcranial magnetic stimulation on the contralateral side; and Group E: Patients with non-massive cerebral infarction will be given high-frequency (10–20 Hz) repetitive transcranial magnetic stimulation on the affected side. The primary outcome measure of this trial is a modified Ashworth scale score from 1 day before treatment to 12 months after treatment. Secondary outcome measures include Fugl-Meyer Assessment of Lower Extremity, Visual Analogue Scale, modified Barthel index, and Berg Balance Scale scores for the same time as specified for primary outcome measures. The safety indicator is the incidence of adverse events at 3–12 months after treatment. We hope to draw a definite conclusion on whether there are differences in the safety and efficacy of low- or high-frequency repetitive transcranial magnetic stimulation combined with botulinum toxin type A injections in the treatment of patients with post-stroke lower limb spasticity under strict grouping and standardized operation, thereby screening out the optimal therapeutic regimen. The study protocol was approved by the Medical Ethics Committee of the First Affiliated Hospital of China Medical University (approval No. [2021] 2021-333-3) on August 19, 2021. The trial was registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR2100052180) on October 21, 2021. The protocol version is 1.1.

Key Words: Botulinum toxin type A; exercise; lower limbs; muscle spasticity; neural regeneration; rehabilitation training; repetitive transcranial magnetic stimulation; stroke

Introduction

Background of study

Muscle spasticity is one of the causes that impede recovery from hemiplegia-induced limb dysfunction after stroke (Chen et al., 2015; Jia et al., 2017; Pan et al., 2020; Bao et al., 2021; Peng et al., 2021). Lower limb muscle spasticity can cause stiffness of the tendon unit and excessive compensatory activity of the synergistic muscles, thereby leading to muscle pain and abnormal gait (Chen et al., 2018). Current treatments for post-stroke lower limb spasticity mainly include rehabilitation therapy (physical therapy and physical factor therapy), oral and intrathecal administration of antispasmodic drugs, local injections of botulinum toxin, and surgery. These therapies have their own limitations to varying degrees, and a single regimen has no satisfactory effects. Therefore, a combination of multiple methods is often used in clinical practice.

Botulinum toxin (BTX) is a kind of neurotoxin containing high molecular protein produced by Clostridium botulinum. BTX can inhibit the release of acetylcholine from nerve endings, induce muscle relaxation and paralysis, and ease muscle spasticity (Rosales et al., 2011). Local injections of botulinum

Department of Rehabilitation Medicine, the First Affiliated Hospital of China Medical University, Shenyang, Liaoning Province, China

*Correspondence to: Ai-Hua Xu, PHD, ahxu@cmu.edu.cn.

https://orcid.org/0000-0002-2447-3692 (Ai-Hua Xu)

How to cite this article: *Shao Y, Yang Y, Sun YX, Xu AH (2022) Different frequencies of repetitive transcranial magnetic stimulation combined with local injection of botulinum toxin type A for post-stroke lower limb spasticity: study protocol for a prospective, single-center, non-randomized, controlled clinical trial. Neural Regen Res 17(11):2491-2496.*

toxin type A (BTX-A), a kind of BTX, can quickly and effectively reduce muscle tone and improve muscle spasticity. In recent years, BTX-A has been widely used for clinical treatment of increased muscle tone induced by upper motor neuron injury (Gupta and Addison, 2020). However, BTX-A only acts on the neuromuscular junction, and cannot thoroughly improve muscle facilitation induced by damage to the brain. Moreover, its effects last for only a short time. Most patients will experience increased muscle tension 3 months after injections. Therefore, a single injection of BTX cannot completely solve the increased muscle tone of the limbs after stroke.

Repetitive transcranial magnetic stimulation (rTMS) is a safe new, non-invasive, physical factor therapy that is often used in post-stroke rehabilitation (Smith and Stinear, 2016; Liao et al., 2017; Kim and Yim et al., 2018; Du et al., 2019; Xu and Sun, 2020). Its main principle is to generate magnetic pulses through electromagnetic induction that enter the cerebral cortex through the skull and reorganize the brain network by regulating the excitability of the brain compartments. A positive effect is then produced in the brain areas related to the regulation of movement, emotion, and cognition, thus improving patient's movement, mood, and cognition. Studies have found that high-frequency rTMS has a facilitating effect on cortical excitability while low-frequency rTMS has an inhibitory effect on the cortex (Di Lorenzo et al., 2013). Low-frequency rTMS can inhibit excitability of the contralateral cortex, while high-frequency rTMS can improve excitability of the affected cortex, both of which can improve affected limb spasticity in stroke patients (Málly and Dinya, 2008). However, limb spasticity can be aggravated if high-frequency rTMS improves excitability of the contralateral cortex (Naro et al., 2017). A meta-analysis reported in 2018 failed to confirm the therapeutic effects of rTMS on post-stroke limb spasticity (Xiang et al., 2018). The meta-analysis reported by Fisicaro et al. (2019) found that high-frequency rTMS can improve limb muscle spasticity, motor function, limb flexibility, balance function, aphasia, and depression in stroke patients. However, there is insufficient evidence for low-frequency rTMS to treat spasticity. Therefore, no definite consensus has been achieved on the clinical safety and efficacy of low- or high-frequency rTMS in the treatment of muscle spasticity after stroke. The latest research shows that low-frequency rTMS combined with local injections of BTX-A is more effective than local injection of BTX-A alone in treating post-stroke limb spasticity (Wang et al., 2016; Tao and Wei, 2018). However, due to a small sample size, the power of the test is low. More clinical trials are warranted to verify actual treatment effects. This trial therefore intends to investigate differences in the safety and efficacy of different frequencies of rTMS combined with local injections of BTX-A in the treatment of lower limb spasticity after stroke, and to screen out the optimal regimen for the treatment of lower limb spasticity after stroke.

Study objective

Our objectives in this study were threefold: (a) verify whether the effectiveness and safety of local injections of rTMS combined with BTX-A are better than the use of rTMS or BTX-A alone on lower limb spasticity after stroke; (b) verify whether differences exist in the effect and safety of low-/high-frequency rTMS combined with BTX-A injection in the treatment of lower limb spasticity after stroke; and (c) summarize the optimal regimen for lower limb spasticity after stroke.

Methods/Design

Study design

This is a prospective, single-center, open, non-randomized, parallel, controlled clinical trial. This trial will enroll 150 patients with post-stroke lower limb spasticity and attempt to investigate the differences in the safety and efficacy of varying frequencies of rTMS combined with BTX-A in the treatment of post-stroke lower limb spasticity.

An ethics approval (approval No. [2021] 2021-333-3) was obtained from the Medical Ethics Committee of the First Affiliated Hospital of China Medical University on August 19, 2021 (**Additional file 1**). Study protocol version 1.1 was registered with the Chinese Clinical Trial Registry (registration No. ChiCTR2100052180) on October 21, 2021. All patients signed an informed consent form (**Additional file 2**). The study will comply with all requirements of the 1964 Declaration of Helsinki, as revised in 2013. Administration of BTX-A and its precautions will follow the Chinese Guidelines for the Treatment of Adult Limb Spasticity (Chinese Association of Rehabilitation Medicine, 2015). Transcranial Magnetic Stimulation (TMS) will be conducted in line with the 2014 International Guidelines for Repetitive Transcranial Magnetic Stimulation Treatment (Lefaucheur et al., 2014). The safety monitoring committee will be responsible for reviewing the data about the safety and efficacy of the unblinded method obtained in this trial every 3 months.

The trial flow chart is shown in **Figure 1**, and the time schedule of outcome measures is shown in **Table 1**. The protocol was written according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist (Chan et al., 2013; **Additional file 3**).

Recruitment

The subjects will mainly be recruited from the Department of Rehabilitation Medicine of the First Affiliated Hospital of China Medical University, which focuses on post-stroke rehabilitation training and treatment. Therefore, the source of patients is sufficient to ensure the number of patients required for the trial. Leaflets providing recruitment information will be distributed among patients with post-stroke lower limb spasticity admitted to the Department of Rehabilitation Medicine of the First Affiliated Hospital of China Medical University. In addition, information about recruitment will also be posted on the hospital's bulletin board. Preferential conditions for participating in the trial are that during the follow-up period, travel expenses, registration fees, various laboratory and imaging examination expenses may be reduced or covered. Professional follow-up visits will be conducted by neurosurgeons at the First Affiliated Hospital of China Medical University. Patients or their families who are interested in this trial can contact the principal investigator by telephone, E-mail or WeChat through their attending physicians. Eligible patients who are fully informed of the benefits and risks of the trial will be included if a written informed consent is given prior to trial inception. Attending physicians will collect and monitor their patient's medical history, symptoms, signs, imaging, and pathological results.

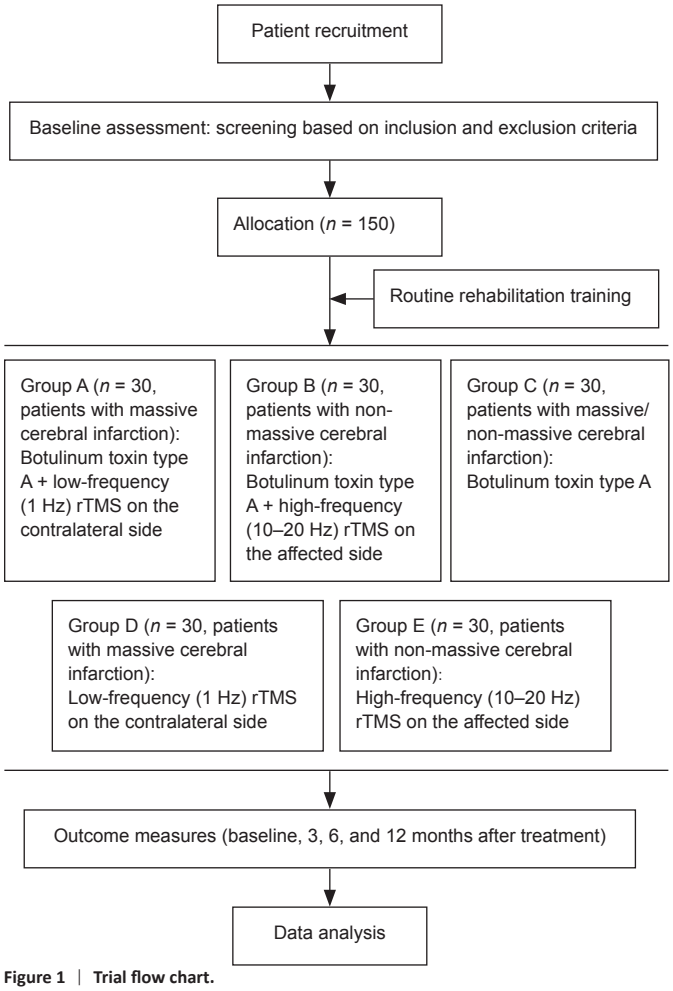


Figure 1 | **Trial flow chart.**
TMS: Transcranial magnetic stimulation.

Table 1 | **Timing of outcome measures**

Items	Baseline (1 d preoperative)	3 mon postoperative	6 mon postoperative	12 mon postoperative
Primary outcome				
Modified Ashworth scale	×	×	×	×
Secondary outcomes				
Fugl-Meyer Assessment of Lower Extremity	×	×	×	×
Visual Analog Scale	×	×	×	×
Berg Balance Scale	×	×	×	×
Modified Barthel Index	×	×	×	×
Safety indicator				
Incidence of adverse events		×	×	×

Inclusion and exclusion criteria

Inclusion criteria

Patients will be determined to be eligible if they meet the following conditions:

(1) After the first onset of stroke, unilateral limb paralysis is diagnosed and confirmed according to the requirements of Stroke: National Clinical Guideline for Diagnosis and Initial Management of Acute Stroke and Transient Ischemic Attack (TIA), as well as the requirements of the 2018 Chinese Acute Ischemic Stroke Diagnosis and Treatment Guidelines issued by Neurology Branch of the Chinese Medical Association and cerebrovascular disease group of Neurology branch of Chinese Medical Association (2018).
(2) They have been diagnosed as having large-area cerebral infarction: In 2017, a Chinese expert consensus on the definition of massive cerebral infarction was launched by the Neurocritical Care Cooperation Group of the Neurology Branch of the Chinese Medical Association, and Neurological Intensive Care Committee of the Neurology Branch of Chinese Medical Association (2017). According to this consensus, CT and MRI of the brain indicate regardless of ipsilateral anterior cerebral artery/posterior cerebral artery infarction in the blood supply area, a massive cerebral infarction should be confirmed if the infarct area is not less than 2/3 of the blood supply area of the middle cerebral artery (Neurocritical care cooperation group, neurology branch, Chinese Medical Association, Neurological intensive care committee of neurologist branch of Chinese Medical Association, 2017). In addition, if the patient with ischemic stroke presents with head-eye separation, gaze disturbance, aphasia and hemiplegia, hemidysergia, and hemianopia, it should be highly suspected of massive cerebral infarction.
(3) They are at least 18 years old.
(4) Scores 10–20 points according to the NIH Stroke Scale (NIHSS) scoring (Kwah and Diong, 2014).
(5) Within 1–6 months after the onset of stroke, there is at least one spastic muscle group on the affected side, which is assessed as having grade 2 lower limb spasticity according to the modified Ashworth scale.
(6) They have the ability to walk independently (with or without walking aids);
(7) They have experienced poor outcomes or severe adverse reactions after oral anticonvulsants.
(8) Patients and his/her family members sign an informed consent form before treatment.

Exclusion criteria
Patients will be excluded if they meet one of the following conditions:
(1) They demonstrate contraindications to rTMS treatment have metal implants such as internal pacemakers and intracranial scaffolds, and/or a history of epilepsy.
(2) They demonstrate contraindications for BTX-A treatment: hypersensitivity and local infection at the injection site.
(3) They have received a local injection of BTX-A within 3 months.
(4) They have a consciousness disorder, and cannot comply with corresponding treatment.
(5) They have severe craniocerebral injury and are at a high risk of seizures;
(6) Pregnant and lactating women.
(7) They have other dystonia diseases, such as myasthenia gravis and Lambert-Eaton syndrome.
(8) They have other acute or chronic diseases and mental illnesses and are therefore not suitable to participate in this clinical trial.

Termination criteria
The principal investigator has the right to terminate the study at any time. Reasons for terminating the study include, but are not limited to, the following: continuation of the study may harm the relevant rights and interests of a certain number of subjects.

Allocation
According to the non-random grouping method, five different treatments will be given to patients with post-stroke lower limb spasticity enrolled in the Outpatient Department of the First Affiliated Hospital of China Medical University. Patients scheduled for treatment and their relatives will be asked whether they agree to participate in the trial. In this clinical trial, the patients who agree to participate in the trial will be assigned into five corresponding treatment groups at a ratio of 1:1, with 30 cases in each group.

Blindness
Collectors and evaluators will be unaware of the study protocol.

Sample size calculation
Based on previous literature and clinical experience, the researchers assume that during the 12-month follow-up period, the average modified Ashworth scale score will be 1.2 points in group A and 1.7 points in group C. Standard deviation is set to be 0.5. Assuming 90% power and two-sided α = 0.05, two-sample *t*-test power analysis of the modified Ashworth scale scores will be conducted if a sample size is determined to be no less than n = 23 in group A and group C using PASS 15.0 software. Considering a 20% dropout rate, n = 28 per group will be included as per 1:1 parallel allocation principle. A total of 140 cases will be included in the five groups. Considering the annual number of patients with lower limb spasticity after stroke admitted and treated at the First Affiliated Hospital of China Medical University, the sample size is ultimately determined to be 30 patients per group (150 patients in total) in this trial.

Interventions
Overall intervention plan
All inducted patients will receive routine rehabilitation training and be divided into five groups according to the area of cerebral infarction and treatment methods, with 30 patients in each group. Specific intervention measures are as follows:
Group A: Patients with massive cerebral infarction will be given local injections of BTX-A + low-frequency rTMS (1 Hz) on the contralateral side;
Group B: Patients with non-massive cerebral infarction will be given local injections of BTX-A + high-frequency rTMS (10–20 Hz) on the affected side;
Group C: Patients with massive/non-massive cerebral infarction will be given local injections of BTX-A;
Group D: Patients with massive cerebral infarction will be given low-frequency rTMS (1 Hz) on the contralateral side;
Group E: Patients with non-massive cerebral infarction will be given high-frequency rTMS (10–20 Hz) on the affected side.

Rehabilitation training
All patients will receive daily routine rehabilitation training, including walking and gait training, proprioceptive neuromuscular facilitation, coordination function training, balance control training, sports re-learning techniques, and activities of daily living. Training time will be no less than 30 minutes per day.

Local injections of BTX-A
(1) Determine the spastic muscles (the main muscles of the lower limbs include posterior tibialis, semitendinosus, semimembranosus, hip adductor and gastrocnemius, etc.) through surface electromyography (EMG). Surface EMG signal, trigger signal and video signal will be simultaneously recorded during the surface EMG (Mega, Finland), and the surface EMG signal will be filtered using a 10–200 Hz filter and collected after full-wave rectification.
(2) According to the Chinese Guidelines for the Treatment of Adult Limb Spasticity with Botulinum Toxin (Chinese Association of rehabilitation medicine, 2015), for muscles with horizontally parallel muscle fibers, several sites across the muscle belly will be selected for BTX injection; for muscles with longitudinal muscle fibers, several sites will be longitudinally selected on the muscle belly for BTX injections. BTX-A for injection (Allergan Inc., Irvine, CA, USA), 100 U, will be dissolved in 2 mL of normal saline, and injected at multiple points on the spastic muscle belly using reverse traction and finger-pressing method. As per the size of the muscle group and the severity of spasm, 10–15 sites will be selected on each muscle group with an interval of 2 cm, and a dose of no more than 50 U per site will be injected, with the total dose not exceeding 600 U in adults.
(3) Repeat injections at the same dose will be performed every 3 months until the 12th month during the follow-up.

rTMS treatment
(1) Equipment: Transcranial magnetic stimulator (O-SELF Medical Technology Co., Ltd., Wuhan, China), equipped with a circular coil, and the peak intensity of pulsed magnetic field is 3.0 T.
(2) Stimulation parameters and methods: low-frequency stimulation frequency is 1 Hz, high-frequency stimulation frequency is 10–20 Hz, and stimulation intensity is 90% resting motor threshold (RMT). At least 5 out of 10 stimulations at the optimal stimulation point have a motor evoked potential (MEP) amplitude ≥ 50 μV, which is determined as RMT. The low-frequency stimulation site is set at the primary motor cortex area (M1 area) of the contralateral side, and the stimulation intensity is 20–30%, 100% RMT, 1000 pulses. The high-frequency stimulation site is set as the motor area of the affected side (M1 area), and the stimulation intensity is 20–30%, 100% RMT, and 1000 pulses.
(3) Time of treatment: 20 minutes per day, 5 days a week, for 4 weeks of treatment in total.

Outcome measures
Primary outcome measure
The modified Ashworth scale (MAS) score will be used to assess muscle tension. To evaluate the severity of muscle spasticity: the scores from low to high are: 0, 1, 1+, 2, 3, and 4. "1+" can be set to 1.5 points for calculation. A higher score indicates more extensive muscle tension and more severe muscle spasm (Ewoldt et al., 2016; Flanigan et al., 2020). Evaluation time points: baseline, 3, 6, and 12 months after intervention.

Secondary outcome measures
(1) Fugl-Meyer Assessment of Lower Extremity will be used to assess lower limb motor function, with a total score of 34 points. A higher score indicates stronger motor ability in the lower limbs (Crow et al., 2014). Evaluation time points: baseline, 3, 6, and 12 months after intervention.
(2) Visual Analogue Scale (VAS) score (range, 1–100) will be used to assess the pain of the affected lower limbs. A higher score indicates more severe pain (Lefaucheur et al., 2004). Evaluation time points: baseline, 3, 6, and 12 months after intervention.
(3) Modified Barthel index (MBI) score will be used to assess quality of life: 100 points indicates normal; ≥ 60 points indicates an individual's basic independence in daily life; 41–59 points indicates moderate dysfunction, i.e., an individual requires help with personal care needs in everyday life; and 21–40 points indicates that an individual is unable to be independent in daily life (Khedr et al., 2005). Evaluation time points: baseline, 3, 6, and 12 months after intervention.
(4) Berg Balance Scale (BBS) score will be used to assess the patient's balance ability: a score of < 40 indicates a risk of falling; a score of 0–20 indicates the need for wheelchairs because of limitations to walking; a score of 21–40

indicates the use of aid during walking; and a score of 41–56 points indicates walking with no assistance (Wu et al., 2020). Evaluation time points: baseline, 3, 6, and 12 months after intervention.

Safety indicator

Incidence of adverse events: Adverse events include seizures and convulsions, scalp tingling, burning sensation, headache, tinnitus, local muscle weakness, autonomic dysfunction, skin rash, and allergic reaction. The incidence of adverse events = (number of patients with adverse events/total number of patients) × 100%. Measure time point: 3, 6, and 12 months after intervention.

Adverse events

Judgment criteria for adverse events

Adverse events include all unexpected clinical manifestations (any unexpected/unfavorable symptoms, signs, diseases or abnormal testing results) that occur after treatment, regardless of whether they are related to the medications administered.

Recording and reporting of adverse events

Researchers should record in detail any adverse events that occur including descriptions of adverse events and all related symptoms, time of occurrence, severity, duration, measures and final outcomes (disappear, relief, persist, etc.).

(1) During treatment, any uncomfortable reactions that subjects complain about or abnormal changes in objective laboratory indicators should be faithfully recorded, including the severity, duration, treatment measures, and outcomes.
(2) Clinicians should comprehensively evaluate the relationship between adverse events and trial drugs according to a five-level classification: definitely related, possibly related, possibly irrelevant, definitely irrelevant, and undeterminable. "Definitely related," "possibly related," and "undeterminable" are all classified as adverse events related to trial drugs.
(3) The incidence of adverse events will be calculated when the sum of these three values acts as the numerator, and the total number of subjects used for safety evaluation is used as the denominator.

Statistical analysis

Principal analysis and sensitivity analysis

The principal analysis will be carried out in the intention-to-treat population, including all patients regardless of the treatment received. Sensitivity analyses will be performed in the as-treated population, with multiple imputations to compensate missing data during the follow-up (Little et al., 2012).

Data description

No interim analysis will be performed in line with the basic principles of intention-to-treat analysis. Statistical analysis will be performed by statistical experts using SPSS 24.0 software (SPSS, IBM, Armonk, NY, USA). Measurement data will be expressed as the mean, standard deviation, median, minimum and maximum value, and interquartile range. Count data will be expressed as the number of cases and percentage.

Analytical methods

Incidence of adverse events will be compared using Pearson χ^2 test or Fisher's exact test. MAS score, Fugl-Meyer Assessment of Lower Extremity score, VAS score, MBI score, and BBS score will be compared using one-way analysis of variance combined with the Bonferroni *post hoc* test (normal distribution) or Kruskal-Wallis *H* test (abnormal distribution). Correlation analysis among outcome indicators will be conducted using Pearson correlation analysis (normal distribution) or Spearman correlation analysis (abnormal distribution). Repeated measures analysis of variance and analysis of covariance will be used to compare the differences in scores between groups during follow-up, investigate the influence of group effects and time effects on the outcome, and analyze the differences in main effects and interaction effects. In this trial, effects of three methods on patient outcomes during the follow-up will be often compared; therefore, the generalized estimation equation will be used to fit the logistic regression model or the general linear regression model to screen the risk of different variables related to outcome indicators at different time points. Inspection level will be set at $\alpha = 0.05$ (two-sided). The statistical methods of this study were reviewed by the statistician from the First Affiliated Hospital of China Medical University.

Data sets

All demographic data at baseline will be analyzed based on the full analysis set, and safety evaluation will be conducted based on the safety set. All clinical indicators for reporting the effectiveness of the trial will be analyzed by the full analysis set and per-protocol set. For the population that meets the per-protocol set analysis, point estimates and 95% CI of each effectiveness index will be calculated, and the difference of each effectiveness index among groups will be also calculated, to determine the clinical effective rate and benefit.

Data collection and management

Data recording

Medical records as original clinical data will be fully collected and recorded in the case report form (CRF) in a timely, correct, complete, clear, standardized, and truthful manner. Researchers must ensure that all the data are true, complete, and accurate. All entries in the CRF should be filled in, empty or missing entries are not allowed (spaces without records should be crossed out). When making any corrections, only lines can be crossed, and the modified data should be marked with the reason, signed and dated by the recorder. Original records must not be erased or overwritten.

Data management

All data related to the trial will not be shared unless they are approved by the principal investigator. All original data, files, experimental reports, summary reports and results of the clinical trials should be stored in the Electronic Data Capture system. All of these should be stored in an orderly manner by the archives room and be easily obtained for quick retrieval. A specific person should be appointed as responsible for management of the archives room. No entry into the archives room can be allowed without approval. Data stored or referenced in the archives room should be properly indexed for easy retrieval. To protect the privacy of subjects, the subject's name should not appear on the CRF. The researchers should confirm and record the subject's identity as per the code of each subject.

Data monitoring

Inspectors will be responsible for supervising whether the study procedures comply with relevant laws and regulations, Good Clinical Practice (GCP) and the study protocol; whether all CRF forms are consistent with the original files and are filled in correctly and completely; and whether there are any data errors or omissions. Inspectors need to repeatedly check the contents of the eCRF with the original files in sequence to ensure that the data in the eCRF are consistent with the original data. This process is also called source data verification (SDV).

Protocol deviation

All requirements specified in the study protocol must be rigorously executed. Any intentional or unintentional deviation or violation as per the study protocol and GCP principles will be classified as a deviation or violation of protocol. During the inspection process, researchers or inspectors will complete the record for study deviation, including the time of discovery, and the time, reason, process, and treatment measures of the event, if a deviation from protocol is detected. The record must be signed by the researchers and submitted to the ethics committee.

All articles and reports related to this study can only be published after consent has been obtained from the principal investigators.

Ethical considerations

Ethical approval

This clinical trial must comply with the relevant requirements of the *Declaration of Helsinki* (2008 version). The study protocol was approved by the Institutional Review Board of the First Affiliated Hospital of China Medical University (approval No. [2021] 2021-333-3) on August 8, 2021. During the clinical trial, the study protocol should be submitted to the Institutional Review Board and filed after certain modifications. Researchers have a duty to regularly submit interim trial reports as per the relevant requirements of the Institutional Review Board, and the Institutional Review Board should be notified of trial completion.

Informed consent

All enrolled patients or family members will participate in the trial voluntarily. All patients should be fully informed of the trial procedures and sign an informed consent form. The study protocol should be confirmed to maximally protect the rights, safety, and health of the subjects prior to inception of the clinical trial, which is a primary principle of the trial. The study protocol must be reviewed and approved by the Institutional Review Board with an approval opinion before implementation.

Revision of the protocol

No one but the principal investigator can amend the study protocol. Any necessary changes in the protocol must be made in the form of a protocol revision, and must be submitted to the Institutional Review Board for approval or filing after obtaining the signature and approval of the principal investigator. Details regarding any revisions made previously should be explained in the protocol.

Dissemination

The final study results will be disseminated through presentations at scientific academic conference and/or publication in a peer-reviewed journal.

Confidentiality

Confidentiality will be maintained during the process of data collection and use, and will comply with the relevant laws and regulations protecting patient privacy. Subjects will provide consent for the collection of their personal data and retain the right to review their personal data and request modifications of incorrect or incomplete data. During the trial, the subjects' personal information will be protected from unauthorized disclosure and from accidental or illegal destruction, loss, or alteration. All sponsor personnel who have access to patient data will ensure that it remains confidential.

Compensation
Special standards and methods of compensation should be clarified prior to trial inception. The sponsor will provide patients with "clinical trial liability insurance," which will cover the cost of treatment and financial compensation for adverse events related to the trial. When applying for compensation, the investigator will determine the relevance of any adverse events to the tested drugs, and claims will be covered for adverse events that are considered to be "relevant" to the trial.

Discussion

The choice of rTMS stimulus frequency
Rehabilitation treatment using rTMS is inseparable from the choice of parameters, among which, the stimulus frequency is particularly important (Sasaki et al., 2013; Gunduz et al., 2014). Generally, 1 Hz is used as the limit for dividing high and low frequencies. A stimulus frequency > 1 Hz is called high frequency, and ≤ 1 Hz is low frequency (Lefaucheur et al., 2014). A meta-analysis showed that both low- and high-frequency rTMS can improve muscle spasticity (Gunduz et al., 2014). However, high-frequency rTMS can increase the excitability of the cortex and induce seizures in patients; therefore, for safety reasons, low-frequency rTMS is usually chosen to reduce its inhibitory effect on the contralateral cortex transcallosum (Mally and Dinya, 2008; Blumberger et al., 2012).

TMS can treat spasticity by modulating the excitability between brain regions and remodeling the brain network (Rivera-Urbina et al., 2017). Currently, there are two hypotheses about brain network alterations after stroke. One is the inter-hemisphere competition model, which is used to describe inter-hemispheric activity after stroke with a larger structural preservation area, that is, the brain activity on the affected side is decreased, and the brain activity on the contralateral side is relatively increased, which causes the contralateral brain region to competitively inhibit the activity of the affected brain region. The other hypothesis is an alternative model, which is used to describe the inter-hemispheric activity with a smaller structural preservation area, that is, the fibrous connection between the contralateral and affected hemispheres is enhanced, replacing the function of the hemiplegic limb (Di Pino et al., 2014). Based on the above theory, low- and high-frequency TMS have been used to inhibit cortical excitability on the contralateral and affected sides for treating post-stroke lower limb spasticity, respectively. Both therapeutic options can improve patients' spasticity (Mally and Dinya, 2008). Another study has found that high-frequency excitement of the contralateral cortex can aggravate spasticity in patients (Naro et al., 2017). In short, inhibiting the contralateral brain activity has no clear effect in the treatment of spasticity. The therapeutic effect of low- versus high-frequency stimulation and mechanisms by which TMS relieves spasticity remain to be further elucidated. In this trial, MAS score will be the primary outcome measure for evaluating muscle spasticity, which can effectively assess the patient's lower limb spasticity before and after treatment.

The choice of locally injected drugs
Locally injected drugs commonly used for limb spasticity include baclofen, ethanol, phenol, and botulinum toxin. Injection of BTX at the neuromuscular junction of spastic muscles can temporarily suppress spasticity and act as an aid to maximize the therapeutic effect on spasticity during the sustained release period (Rosales et al., 2011). Currently, BTX biologics approved by the US Food and Drug Administration include BTX-A, BTX-B, and prabotulinum toxin A xvfs. BTX is safer and more effective than baclofen, ethanol, and phenol for injection (Lui et al., 2015). However, some patients will develop BTX resistance and poor outcomes after a period of receiving injections, which may be related to the production of BTX neutralizing antibodies. The production of neutralizing antibodies is related to high doses, short intervals, and multiple injections (Dressler and Hallett, 2006). However, studies have shown that neutralizing antibodies may not necessarily result in BTX tolerance. Differences in the efficacy of BTX injection may also be related to target muscle, injection dose, and injection technique. Specific reasons and mechanisms still need to be further elucidated (Mathevon et al., 2019). Overall, BTX-A will be selected for local injections in this trial.

Trial limitations
Due to the limitations of the hospital's practical conditions and operational particularity of the therapeutic regimen, neither randomization nor blindness of patients and doctors can be achieved in this trial. A follow-up period of 3–12 months is relatively short. Longer-term follow-up effects have yet to be evaluated. The outcome measures are mostly functional scoring indicators, which are relatively single, and there is a lack of electrophysiological indicators (such as motor evoked potentials, H reflex and H_{max}/M_{max}) (Tretriluxana et al., 2013; Yamada et al., 2014; Cakar et al., 2016). All of these may have a certain impact on the accuracy of the trial results.

Conclusion and prospects
In this trial, different frequencies of rTMS combined with BTX-A injections will be used to treat patients with post-stroke lower limb spasticity, which is beneficial for determining the optimal combination treatment and reducing patients' tolerance and poor outcomes due to BTX injection alone. After a period of time, botulinum toxin tolerance and poor therapeutic effect will appear. We hope that a combination therapy with the best curative effect can be selected to effectively improve lower limb spasticity in stroke patients.

Trial Status

The trial was registered with the Chinese Clinical Trial Registry (registration No. ChiCTR2100052180) on October 21, 2021. The trial started enrolment on December 1, 2021 and is expected to be completed by December 1, 2022. Analysis on primary outcome measures will be completed on December 10, 2022. The trial will end on December 31, 2023. The protocol version is 1.1.

Author contributions: *AHX designed the trial and was responsible for the manuscript. All authors participated in recruitment, data collection and analysis and approved the final version of this paper.*
Conflicts of interest: *The authors have no conflicts of interest to declare.*
Availability of data and materials: *All data generated or analyzed during this study are included in this published article and its supplementary information files.*

Additional files:
Additional file 1: *Ethical Approval Documentation (Chinese).*
Additional file 2: *Informed consent form (Chinese).*
Additional file 3: *SPIRIT checklist.*

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